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OXYGEN CONCENTRATION AS A LIMITING FACTOR IN THE RESPIRATORY METABOLISM OF *PLANARIA AGILIS*.

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Living organisms have been conveniently grouped into obligate aërobes, facultative aërobes, facultative anaërobes and obligate anaërobes. The criterion for this classification is fundamentally the relation of organisms to the *concentration* of free oxygen. Free oxygen by its concentration effects on the metabolism acts as a limiting factor in determining animal and plant distribution. While the rôle of oxygen concentration in the life of lower plants and animal parasites has been known in a general way for a long time, yet the importance of differences in oxygen concentration as a factor determining the differences in distribution of many invertebrate animals, which we would ordinarily consider aërobic, has not received the emphasis which it deserves. The grouping of organisms into the above four groups is merely arbitrary, for from the small amount of data available it is clear that some organisms stand in an intermediate position between the above groups.

Since the rate of respiration is correlated to such conditions as state of nutrition, size and age, regeneration, cell division and irritability, it is logical to suppose that by comparing the effects of different oxygen concentrations in the different states of nutrition, age, regeneration and so on, valuable information might be obtained which would throw further light on the relation of respiration to this group of correlated processes.

In the present paper three questions will be considered in so far as they apply to *Planaria agilis*: (1) What is the quantitative relation between oxygen concentration and the rate of oxygen consumption? (2) What is the effect of the state of nutrition upon the relation of oxygen concentration to rate of oxygen

consumption? (3) What is the relation of oxygen concentration to the rate of carbon dioxide production and irritability in *Planaria agilis*, which ordinarily would be considered aërobie?

THE RELATION OF OXYGEN CONCENTRATION TO THE RATE OF OXYGEN CONSUMPTION.¹

The change in rate of oxygen consumption by *Planaria agilis* while it is gradually consuming a given quantity of oxygen dissolved in a given volume of water in a stoppered bottle is shown in the curve in Fig. 1. The data for the curve were obtained as follows: Twenty-one bottles of equal volume were filled with

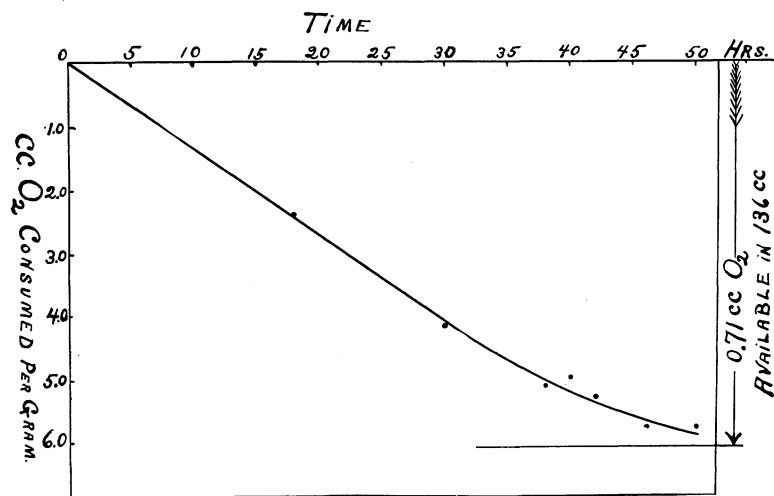


FIG. 1.

water containing the same amount of oxygen. Ten animals of equal size and the same history were placed in each bottle. At the end of different time periods as indicated in the curve three bottles were analyzed for oxygen content and the average taken as the value which is plotted on the ordinate. The animals had starved for over ten days previous to the experiment and therefore had practically a constant rate of respiratory metabolism as shown by Allen ('19, II.).

¹ The following is a part of certain studies on respiratory metabolism made possible by a grant from the research fund of the graduate school.

The animals which were used in the experiments reported in this paper were kept at 20° C. during the experiments, and for from two days to two weeks previous to each experiment in order to avoid any acclimation effects due to temperature. The curve shows that the rate of oxygen consumption was constant up to about thirty-eight hours, at which time the oxygen concentration was equal to about one fourth to one sixth of the oxygen concentration of air-saturated water at 20° C. When lower oxygen concentrations were approached the rate decreased. An unused residue of oxygen remained at the end of the experiment. This would have been quickly consumed if the original rate of oxidation had been maintained.

In this method of experiment the possibility that acclimation of the rate of oxygen consumption to lower oxygen concentrations occurs is not excluded. Therefore the following procedure was adopted. If the animals are subjected *suddenly* to definitely known low oxygen concentration and the time period during which they are left in this low oxygen concentration is made brief, then acclimation will probably be avoided. The procedure was as follows. Twenty-eight lots of worms with twenty worms in each lot were selected. All the animals were very closely alike in size, history, etc. The experiment was continued for three successive periods of three hours each. During the first period each one of the lots of animals was placed in a bottle containing air-saturated water. The rate of oxygen consumption per gram worm per hour for this period was determined. At the end of the first period the twenty-eight lots of animals were transferred directly into a second set of twenty-eight bottles containing different but known amounts of oxygen per 136 c.c. water. The animals were now left in these bottles for a second period of three hours. There were fourteen different known concentrations of oxygen. Two bottles were used for each concentration in order to increase the accuracy of the determinations. The fourteen different concentrations of oxygen were as follows: 0.029 c.c., 0.048 c.c., 0.085 c.c., 0.106 c.c., 0.127 c.c., 0.149 c.c., 0.168 c.c., 0.177 c.c., 0.215 c.c., 0.265 c.c., 0.280 c.c., 0.711 c.c., 1.420 c.c. and 1.840 c.c. per 136 c.c. of water.

In this series 0.711 c.c. of oxygen per 136 c.c. water represents the concentration at air saturation at 20° C. The different concentrations of oxygen were obtained by properly mixing known volumes of water of high oxygen content with oxygen-free water.

At the end of the second period of three hours, each lot of worms was again immediately transferred to a bottle containing air-saturated water and left for a third period of three hours. The rate of oxidation per gram per hour during this third period was again determined.¹

The only results from this experiment with which we are concerned at this time are the rates of oxygen consumption per gram per hour during the *second* period in different concentrations of oxygen. These amounts of oxygen corresponding to the fourteen different concentrations of oxygen are represented by the points on the curve in Fig. 2. Each point is an average of the deter-

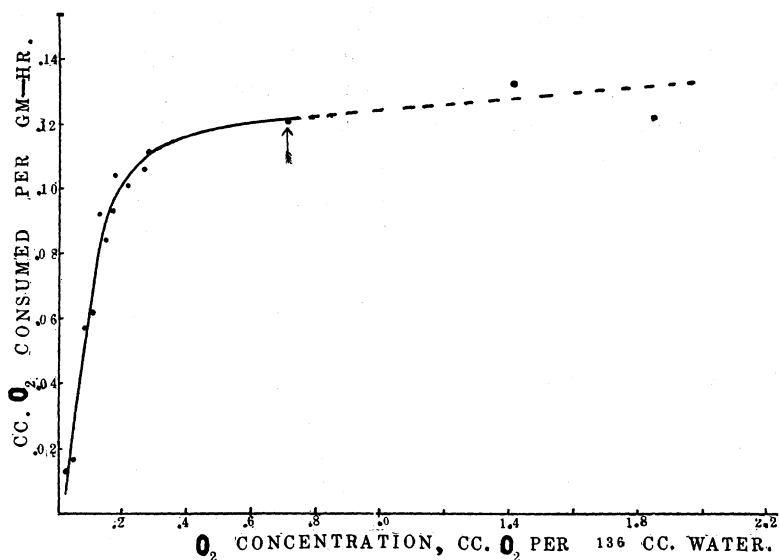


FIG. 2.

minations on two lots of worms. From the curve in Fig. 2 it will be seen that the oxygen concentration which begins definitely

¹ Many precautions and details of technique must be observed in such a relatively complex experiment so that the three-hour period was selected as most suitable. The writer is indebted to Miss Edna Wolf for valuable help in many of the experiments reported in this paper.

to act as a limiting factor on the rate of oxygen consumption lies somewhere around one third and one fourth of the concentration at air saturation, a little higher than that indicated by the curve in Fig. 1. Whether or not the respiratory mechanism of *Planaria* can actually adjust itself by maintaining to some extent a constant rate of oxygen absorption at oxygen concentrations below that which is limiting in such an experiment as the one given above, is an interesting question and will be referred to again.

The question arises as to whether or not *Planaria* can remove the last traces of dissolved oxygen. In general the impression obtained from many experiments of one type or another was, that the very last traces of oxygen could in general not be removed.³

The slope of the part of the curve shown by the interrupted line is the part which was left in some doubt in this experiment. Hence several separate tests were made on different lots of animals in order to determine more definitely whether high concentrations of oxygen do increase the rate of oxygen consumption. In each of the experiments two identical lots of animals were tested at air saturation and high oxygen concentration. The following are the results of three separate tests on different lots of worms.

Concentration of Oxygen at Beginning.		Oxygen Consumed per Gram per 24 Hours.	
C.c. O ₂ in 136 c.c. water.			
Exp. I.	{ 0.820 1.996	(Air Sat.)	{ 4.02 4.38
Exp. II.	{ 0.797 1.768		{ 2.92 3.36
Exp. III.	{ 1.320 2.920		{ 3.88 3.74

In general the results from several experiments indicate that there is a slightly higher rate of oxygen consumption at concentrations above air saturation than at air saturation. The approximate amount of this increase is indicated by the slope of the broken part of the curve. The experiment clearly shows that

³ Winkler's method as ordinarily used is however not sufficiently accurate as a means of giving an absolute answer to this question, for iodine from potassium iodide is slowly set free by an excess of acid.

oxygen concentration may act as a limiting factor in the rate of oxygen consumption and shows also what are the quantitative relations.

This result is similar to those reported by Henze (10) for *Anemonia sulcata*, by Thunberg ('05) for *Limax* and *Lumbricus*, and in the leech as found in this laboratory by Miss Wolf. In a previous paper by Lund ('18) it was shown that the rate of oxygen consumption by *Paramecium caudatum* is practically entirely independent of the concentration of oxygen. This relation would be represented in Fig. 2 by a straight line drawn from some appropriate point on the y -axis parallel to the x -axis. Evidently the oxidation reactions in *Paramecium* and *Planaria* are quite different in certain respects. This is further shown by the fact that the inhibitory action of KNC on the rate of oxygen consumption in *Planaria* is very marked, as shown by Allen ('19, I.), while a similar inhibitory action on the oxygen consumption in *Paramecium caudatum* could not be found, Lund ('18, II.).⁴

It may well be that we shall be able to find in the results of studies on the relation of oxygen concentration to the rate of oxygen consumption, a key to the solution of the question as to why it is that the action of KNC upon *Planaria agilis* and *Paramecium caudatum* is different in character. Work is under way on this question.

It must be kept in mind that since we do not as yet have any accurate data on the magnitude of the diffusion coefficients of oxygen through the protoplasm of cells and different tissues, it is really impossible to say whether in such a case as *Planaria* for example, the decrease in the rate of oxygen consumption at lower oxygen concentrations is due to slow rate of diffusion of the oxygen into the interior of the animal, which rate is itself a function of the difference of oxygen pressure between the inside and outside of the animal, or whether the difference is actually due to an inherent difference in reaction velocity as expressed

⁴ Various kinds of criticisms of these experiments have been made by Child and Hyman. A careful consideration of the objections offered has however entirely failed to disturb my confidence in the results as given in the experiments, and the correctness of the conclusions drawn from them. A more complete consideration of the criticisms with further experimental data is given elsewhere. Lund ('21.)

for example by the velocity constant in the equation for a bimolecular chemical reaction, Krogh ('19). In the absence of appropriate experimental data further discussion of this question is unprofitable.

Whatever may be the ultimate explanation or cause of the drop in the curve toward the origin of the axes, such curves for different animals nevertheless may turn out to be useful in interpreting the rôle of oxygen concentration as a factor in determining the distribution of the organism, and if further experiment can show that difference in concentration of oxygen between the outside and inside of the animal does not act as the limiting factor, but rather the concentration of oxygen as such in the cells, then such curves will be very important criteria for the differentiation of different types of respiratory metabolism among animals. A careful comparative study of, for example, the terrestrial, semi-aquatic, and mud-dwelling species of earthworms might throw further light on the question.

THE RATE OF OXYGEN CONSUMPTION BY PLANARIA IN AËRATED WATER AFTER LIVING IN WATER OF LOW OXYGEN CONCENTRATION.

Planaria agilis shows a characteristic increase in the rate of oxygen consumption after removal to aërated water from oxygen-free water. This is illustrated in Table I. The experiment was divided into three consecutive test periods. During the first period tests on the seven lots of animals were made in air-saturated water. After eight hours the lots were transferred to a low concentration of oxygen for periods of seven, nine and twelve hours, and at the end of these test periods they were transferred directly into aërated water and the rate of oxygen consumption per gram per hour determined for a third period of four hours. Table I. is a complete statement of the results.

It will be noted that during the third period there is an increase in rate over that in the first period varying from 49 to 85 per cent. There is no proportionality between this percentage increase and the length of time which the animals were subjected to the low oxygen concentration as one might at first expect.

TABLE I.

Planaria starved 26 days before experiment, and kept at 20° C. for 7 days before and during tests. 20 animals in each bottle.

Bottle.	Period I.—8 hours.			Period II.—7–12 hours.			Period III.—4 hours.		
	Air-saturated Water.			Water with Low O ₂ Content.			Air-saturated Water.		
	C.c. O ₂ in 136 c.c.			C.c. O ₂ in 136 c.c.			C.c. O ₂ in 136 c.c.		
		0.713			0.033			0.729	
		0.715			0.034			0.736	
		0.715			0.034			0.732	
	Weight of 20 Worms.	Total O ₂ Consumed.	O ₂ Consumed per gm. per 24 hours.	Length of Period.	Total O ₂ Consumed.	O ₂ Consumed per gm. per 24 hours.	Total O ₂ Consumed.	O ₂ Consumed per gm. per 24 hours.	Per Cent. Increase in Rate of O ₂ Consumption.
	gms.	c.c.	c.c.	hrs.	c.c.	c.c.	c.c.	c.c.	
1	.167	.164	2.95	7	.012	.248	.123	4.41	49%
2	.165	.143	2.61	7	.017	.352	.132	4.77	82%
3	.165	.152	2.76	7	.014	.286	.140	5.08	84%
4	.164	.161	2.93	9	.026	.420	.147	5.36	82%
5	.163	.157	2.90	9	.026	.420	.138	5.10	75%
6	.161	.147	2.73	12	.026	.420	.137	5.08	85%
7	.163	.156	2.83	12	.026	.420	.126	4.64	63%

TABLE II.

Planaria starved 14 days before experiment. Temperature 20° C. 11 days before and during tests. Twenty animals in each bottle. Numbers in the table are averages of three bottles. Limit of experimental error 0.010 c.c. oxygen.

Bottles.	Total Weight of Worms in Grams.	Period I. 6 hours. Air-saturated Water. C.c. O ₂ in 136 c.c. 0.720		Length, Hrs.	Period II. C.c. O ₂ in 136 c.c. Bottles 1 to 12, 0.029 Bottles 13 to 21, 0.062		Period III. 6 hours. Air-saturated Water. C.c. O ₂ in 136 c.c. 0.734		Increase.
		Total O ₂ Consumed.	O ₂ Consumed per Gram per 24 Hours.		Total O ₂ Consumed.	O ₂ Consumed per Gm. Hr.	Total O ₂ Consumed.	O ₂ Consumed per Gm. Hr.	
1, 2, 3....	0.569	c.c. 0.42	c.c. 2.95	1.5	c.c. (0.01 —)	0.2 —	c.c. 0.52	c.c. 3.67	24%
4, 5, 6....	0.608	0.41	2.71	3	0.02	0.25	0.57	3.79	36%
7, 8, 9....	0.567	0.43	3.04	4	0.04	0.42	0.56	3.99	31%
10, 11, 12	0.593	0.40	2.72	6	0.04 +	0.31	0.55	3.69	35%
13, 14, 15	0.572	0.41	2.85	9	0.13	0.60	0.68	4.75	66%
16, 17, 18	0.560	0.45	3.19	16	0.15	0.41	0.74	5.31	66%
19, 20, 21	0.561	0.46	3.31	24	—	—	0.70	5.00	51%

To test further whether the length of time during which the animals lived in low oxygen concentration had any effect on the percentage increase during the third period, the results of an experiment in Table II. are given. The procedure was the same as that in the experiment, Table I., except that a larger number of bottles were used, with a greater range in the length of the second period, the latter varying from 1.5 hours to 24 hours. Roughly speaking, there is a slightly smaller average acceleration of the rate in the lots of worms subjected to low oxygen for the shorter periods than in the lots left in the low oxygen for the long periods. The difference in the increase is in general quite independent of the length of the period of low oxygen.

COMPARISON OF THE RATES OF OXYGEN CONSUMPTION IN
AIR-SATURATED WATER BY STARVED AND FED ANIMALS
IMMEDIATELY AFTER HAVING BEEN SUBJECTED TO
LOW CONCENTRATION OF OXYGEN.

If we assume that the mechanism which determines the rate of oxygen consumption can undergo an adjustment when subjected to low oxygen concentration, in such a way as to compensate for the inhibitory effect of low oxygen on the rate of consumption, then, would it not be possible to test this assumption by comparing the acceleration, in rate of oxygen consumption, after life in low oxygen, of two lots of animals one of which has been previously subjected to repeated periods in low oxygen concentration, while the other has been manipulated mechanically in exactly the same way without subjection to low oxygen previous to the test?

The data in the columns under lot A and lot B in Table III. give the results of one test. Lot A was subjected to very low oxygen during two alternate periods of eight hours each, while lot B was manipulated similarly except that it was kept in aerated water. The average actual increase in rate of oxygen consumption by twenty animals during the third period is 1.08 c.c. oxygen per gram per 24 hours in lot A, while in lot B it is 1.54 c.c. oxygen per gram per 24 hours. The average increase is therefore 33 per cent. for lot A and 51 per cent. for lot B. A comparison of

TABLE III.

Planaria starved 7 days before experiment and kept at 26° C. 3 days before and during tests. Each lot of 80 worms was divided into four groups of 20 each for the tests.

	Lot A (80 worms). <i>Starving.</i>		Lot B (80 worms). <i>Starving.</i>	Lot C (80 worms). <i>Fed.</i>
1 Period 8 hrs.	Low O ₂ concentration.		Air-saturated water.	Air-saturated water.
2 Period 12 hrs.	Air-saturated water.		Air-saturated water.	Air-saturated water.
3 Period 8 hrs.	Low O ₂ concentration.		Air-saturated water.	Air-saturated water.
4 Period 44 hrs.	Air-saturated water.		Air-saturated water.	Air-saturated water. Fed liver at beginning of period.
	Bottle.	O ₂ Consumed per Gram per 24 Hours.	O ₂ Consumed per Gram per 24 Hours.	O ₂ Consumed per Gram per 24 Hours.
Period I. 6 hours c.c. O ₂ in 136 c.c. = 0.611	1	c.c. 3.20	c.c. 2.75	c.c. 4.76
	2	3.30	3.09	4.89
	3	3.26	2.87	5.23
	4	3.31	3.36	4.69
	Avg.	3.27	3.01	4.89
Period II. 10 hours c.c. O ₂ in 136 c.c. = 0.048	1	.37	.408	.42
	2	.37	.396	.43
	3	.37	.392	.42
	4	.40	.430	.38
	Avg.	.38	.40	.41
Period III. 6 hours c.c. O ₂ in 136 c.c. = 0.672	1	4.14	4.61	6.15
	2	4.21	4.50	6.09
	3	4.57	4.37	5.45
	4	4.49	4.73	6.36
	Avg.	4.35	4.55	5.76
Actual average increase.		1.08	1.54	0.87
Increase.		33%	51%	17%

the same set of twenty animals in each of the bottles 1, 2, 3 and 4 for the first and third periods will show that the increase is similar in all the bottles. For lot A it is 0.94, 0.91, 1.31 and 1.18 for bottles 1, 2, 3 and 4 respectively, while for lot B it is

1.86, 1.41, 1.50 and 1.37 for bottles 1, 2, 3 and 4 respectively. It might seem that the previous life in low oxygen did have an effect which resulted in slightly decreasing the acceleration in aerated water. This therefore, so far as it goes, confirms the inference from the comparison of the curves in Figs. 1 and 2 above in respect to the difference in the limiting concentrations of oxygen in the two methods of experiment used in obtaining the two curves.

A second question upon which the experiment given in Table III. was intended to throw light is: What effect does the state of nutrition have upon the magnitude of the increase of oxygen consumption during the third period? The animals of lot B were treated exactly like those in lot C except that those in lot C were fed liver about forty-two hours before the beginning of the first test period. The expected marked rise in oxygen consumption occurs in lot C during the first period. This rise persists during the third period but it will be noted that the increase in rate during the third period over that in the first period is only 0.87 c.c. per gram per 24 hours in the fed lot C as compared to 1.54 c.c. per gram per 24 hours in the starving lot B. The average increase in lot C is 17 per cent. while in lot B it is 51 per cent. This question was tested further by comparing fed and starved animals. Table IV. is self-explanatory. The animals in bottles numbered 5 to 8 were fed beef liver twenty-four hours previous to the experiment. The results again show a greater increase of oxygen consumption by the starved than by the fed animals, during the third period.

It was suspected that the increased rate of oxygen consumption during the third period might be due to increased motor activity by the animals during the third period. Accordingly in an experiment, the results of which are given in Table V., the heads of both starved and fed animals were cut off after feeding, that is, about 24 hours before the beginning of the experiment. All the data are calculated on the basis of the weights of the animals before feeding after subtracting the weight of the heads which were removed after feeding. The results on such decapitated fed and starved animals show the same relations in respect

TABLE IV.

All *Planaria* starved 26 days before experiment. Temp. 20° C. 7 days before and during experiment. 18 animals in each bottle.

		Period I. 6 hours.	Period II. 9 hours.	Period III. 6 hours.	
		O ₂ Concentration in c.c. per 136 c.c. Water.			
		0.672	0.013	0.674	
	Bottle.	Total c.c. O ₂ Consumed During the Period.			
Starved.....	1	0.083	0.005	0.119	
	2	0.106	0.005	0.122	
	3	0.100	0.005	0.116	
	4	0.126	0.010	0.136	
Fed.....	5	0.169	0.012	0.183	
	6	0.178	0.015	0.192	
	7	0.185	0.012	0.202	
	8	0.174	0.017	0.173	
		Average, C.c. Oxygen Consumed per Gram Weight per 24 Hours.			
		Period I.	Period II.	Period III.	Increase.
Starved.....		3.030	0.127	3.650	20%
Fed.....		5.220	0.204	5.540	6%

to the increased rate of oxygen consumption during the third period, as that found in the whole animals used in Tables III. and IV. The absolute increase during the third period, among the different experiments, is not accounted for by any difference in the length of the starvation periods of the animals previous to the experiments, for these were the same. On the other hand, any differences in motor activity which may have occurred in the different experiments do not seem to me to give any adequate physiological explanation of the effect. The magnitude of the effect of movement on the respiratory exchange may be inferred from certain observations on *Planaria agilis* made by Allen ('19, I.). There remains the alternative explanation, that the increase in oxygen consumption after living in low oxygen concentration is due to the oxidation of lower fatty acids or other by-products

TABLE V.

All *Planaria* starved 33 days before experiment. Temp. 20° C. 30 days before and during the tests. One half of the total number of worms were fed beef liver 24 hours before beginning of the tests. *Heads of all the worms were cut off one hour after feeding.* 20 worms in each bottle.

			Period I.	Period II.	Period III.	
			O ₂ Concentration in c.c. per 136 c.c. water.			
			0.752	0.030	0.748	
	Bottle.	Weight of Headless Worms, gms.	Total c.c. O ₂ Consumed During the Period.			
Fed.....	1	0.146	0.207	0.013	0.200	
	2	0.143	0.203	0.008	0.215	
	3	0.139	0.195	0.010	0.196	
	4	0.138	0.212	0.010	0.222	
Starved.....	5	0.132	0.106	0.005	0.142	
	6	0.133	0.125	0.008	0.116	
	7	0.131	0.111	0.002	0.135	
	8	0.135	0.115	0.007	0.127	
			Average, C.c. Oxygen Consumed per Gram Weight per 24 Hours.			
			Period I.	Period II.	Period III.	Increase.
Fed.....			4.34	0.16	4.44	2%
Starved.....			2.57	0.07	2.93	14%

of catabolism which accumulated during the period of life in low oxygen.⁵ In the absence of a complete chemical study of the metabolism of *Planaria* the results await an adequate explanation.

⁵ In his monograph "Respiratory Exchange of Animals and Man," Chapter VI., page 83, Krogh states that, according to Lesser (*Zeitschr. f. Biol.*, vol. 54, pp. 1-17), earthworms after living in the absence of oxygen, when returned to air show an increased rate of oxygen consumption, due to oxidation of accumulated oxidizable residues during the anaerobic period. In fact Lesser found just the opposite and states definitely, pp. 11-12: "Durch Respirationsversuche . . . wurde festgestellt, das der respiratorische Quotient beim Regenwurm . . . in der Erholung nach vorausgangener Anoxybiose erhöht ist gegenüber dem normalen. Es findet mithin in der Erholung eine völlige Verbrennung . . . der früher als Hauptprodukt der Anoxybiose gefundenen Fettsäure nicht statt."

THE RATE OF CO₂ PRODUCTION IN THE
ABSENCE OF FREE OXYGEN.

The rate of carbon dioxide elimination by *Planaria* was determined by the use of a modification of the method described some time ago by Lund ('19). An important improvement in the method was brought about by washing the bottles with a stream of CO₂-free air, after the animals were placed in the bottles, thus doing away with the correction for CO₂ present in the air at the beginning. Where CO₂ production was determined in the absence of oxygen the bottles were washed out with hydrogen. All titrations were performed without removing the stopper from the bottle, by means of the simple expedient of boring two holes, one on each side of the bottle. These holes were fitted with stoppers and titration was carried on through one of these small openings. This procedure simplifies the method and increases its accuracy. The method has been used extensively in this laboratory and has proven itself to be satisfactory, especially when periods of two to three hours or more are used and comparative results are desired.

Table VI. gives the results of an experiment where twelve lots

TABLE VI.

Planaria starved and kept at 20° C. 38 days before experiment. Temperature during experiment 21° C. Twelve bottles with 50 animals in each. Blanks run as controls are not given in the table. 1 c.c. N/100 HCl equivalent to 0.111 c.c. CO₂.

Duration of Test in Hours.		5	10	15
Air. . .	Total c.c. CO ₂ produced per gram during test.	0.645	1.332	2.041
		0.504	1.561	2.241
		Avg. 0.574	1.446	2.141
	Average c.c. CO ₂ produced per gram during last 5 hours of test.	0.57	0.87	0.69
H ₂ . . .	Total c.c. CO ₂ produced per gram during test.	0.708	1.371	2.140
		0.766	1.345	2.077
		Avg. 0.737	1.358	2.108
	Average c.c. CO ₂ produced per gram during last 5 hours of test.	0.73	0.62	0.75

of fifty animals in each lot were used. Six bottles were filled with CO₂-free air while another lot of six bottles were filled with hydrogen. The CO₂ produced by the worms in two bottles from each of the series filled with air and hydrogen respectively was determined at the end of 5, 10 and 15 hours, as shown in the table. The amounts of CO₂ produced during each one of the successive periods of five hours in air was 0.57 c.c., 0.87 c.c., 0.69 c.c. CO₂; and in hydrogen 0.73 c.c., 0.62 c.c. and 0.75 c.c. CO₂. The lots of fifty worms each were of course selected carefully as to size, history, etc., so as to render them comparable. The table shows clearly that no marked difference in the rate of CO₂ elimination occurs in the animals in air and those without free oxygen. All animals were normal at the end of the experiment.

Table VII. is a similar experiment of longer duration. The

TABLE VII.

Planaria starved and kept at 20° C. 11 days before the experiment. Temperature during the experiment 19.5° C. Twenty-five worms were placed in each bottle in 2 c.c. water. Blanks run as controls are not given in the table. 1 c.c. N/100 HCl equivalent to 0.111 c.c. CO₂.

Duration of Test in Hours.		7	14	21	28
Air. . .	Total c.c. CO ₂ produced per gram during test.	0.89	1.65	2.99	4.17
		0.72	1.86	3.45	4.17
		0.80	1.75	3.22	4.17
	Average c.c. CO ₂ produced per gram during the last 7 hours of test.	0.80	0.95	1.47	0.95
H ₂ . . .	Total c.c. CO ₂ produced per gram during test.	0.70	1.84	—	2.95
		0.58	1.46	2.97	3.41
		0.64	1.70	2.97	3.18
	Average c.c. CO ₂ produced per gram during the last 7 hours of test.	0.64	1.06	1.27	0.21

carbon dioxide elimination during the successive four periods of seven hours each is in air 0.80 c.c., 0.95 c.c., 1.47 c.c., 0.95 c.c., while in hydrogen it is 0.64 c.c., 1.06 c.c., 1.27 c.c., 0.21 c.c. A comparison of the condition of the animals at the end of the 7-, 14- and 21-hour periods showed all the animals in hydrogen to have a normal response to light and touch. While at the end of twenty-

eight hours irritability was practically lost in those living in hydrogen. The animals in air were normal. The loss of irritability is clearly correlated to the fall in rate of CO_2 elimination during the last seven-hour period. All animals in this twenty-eight-hour period in hydrogen recovered when removed to air.

Another similar experiment which lasted thirty-eight hours gave similar results. A fall in rate of CO_2 elimination in hydrogen was again associated with loss of irritability and muscular tone. One set of animals in the thirty-eight-hour period only partially recovered when removed to air-saturated water. The controls in air were normal.

It is evident that the loss of irritability and appearance of a condition which closely resembles narcosis is correlated more closely to decrease in rate of CO_2 production than the lack of free oxygen.

An important fact to notice is that the action of KNC and the effect of absence of free oxygen are quite distinct. The experiments above show clearly that the rate of CO_2 elimination is not noticeably changed for hours after free oxygen has been removed. Potassium cyanide in proper concentrations inhibits oxygen consumption to as much as 70 to 80 per cent., as shown by Allen ('19, I.) and confirmed by Hyman ('19), and also markedly inhibits carbon dioxide elimination as found by Child ('19). While the absence of free oxygen does not affect the rate of CO_2 production for many hours, so far as the evidence goes, the return of *Planaria* to air-saturated water from KNC solutions strong enough to cause an inhibition of 50 to 80 per cent. does not result in an increase in the rate of oxygen consumption, after the return to air-saturated water, as is the case after life in the absence of free oxygen. Another fact to be noted is that it has not so far been possible to obtain a complete inhibition of the oxygen consumption in *Planaria agilis* by means of KNC. These differences in the action of KNC and lack of oxygen have not been fully recognized by previous investigators, while it has often been assumed that lack of oxygen and KNC solutions have the same effects.

SUMMARY.

1. Oxygen concentration becomes a limiting factor in the rate of oxygen consumption by *Planaria agilis* at about one third air saturation of water at 20° C.

2. Life in low oxygen concentrations results in an accelerated respiratory metabolism varying from two to eighty-five per cent. when the animals are returned to air. This increase is not proportional to, nor directly dependent upon, the previous length of time which the animals have lived in what practically amounts to an absence of oxygen. Some slight evidence was obtained which indicated that repeated subjection of *Planaria agilis* to low oxygen concentration may result in a small decrease of the acceleration of oxygen consumption when returned to aerated water after living in very low oxygen concentration.

3. The percentage acceleration of oxygen consumption referred to under 2 is more marked in starved than in fed animals.

4. The rate of carbon dioxide elimination continues at practically the same rate in hydrogen as in air, until loss of irritability and muscular tone begins. The loss of irritability in *Planaria agilis* is therefore more closely correlated to change in rate of CO₂ production than to consumption of free oxygen if in fact it is correlated closely to either one.

5. The effect of KNC in inhibiting the oxidations in *Planaria* is not identical with the effects due to the absence of free oxygen.

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